



**Fig. 1**

## Figure 2

Mutation	Exemplary Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	$E_{260}$ M <sup>-1</sup> cm <sup>-1</sup>
2789+5G>A	1	none	invader	TTTGGTTTGCTGTGGCTCCCTTGGAAAGTGAT	330800
2789+5G>A	1	hex	probe/DM	CGCCCGAGGATATTCATGCTCTATTGTG	306500
2789+5G>A	1	none	synthetic target	CAATCTACACATAGACATGAATAATCACTTCCAAAGGAGCCACGACACACCAAA	667000
R1162X	1	none	invader	GTTACCTCTGTGGCATGTCAATGAACCTTAAGACTCT	428000
R1162X	1	hex	probe/DM	CGCCCGAGGAGCTCACAGATCGC	253000
R1162X	1	none	synthetic target	TCAGATCGCAATCTGTGAGCTGAGTCTTAAAGTTTCATTGCATGCCAACAGAGGTAAAC	659000
R347P	4	none	invader	CAGGAAATTGCCGAGTGAACGCCCATGT	306600
R347P	4	hex	probe/ER24	ACGGACGCGGAGGCGACAAATCGAG	318200
R347P	4	none	synthetic target	CTCATCTGCATGTCTTGCCCATGCGGTCACTCGGCAATTTCCCTGGG	488000
1898+1G>A	1	none	invader	GATCTCTCTTTGGTACCTAGATTTTAAACAGAAAAGAAATATTTGAAAGT	619900
1898+1G>A	1	hex	probe/DM	CGCCCGAGGATGTCTTTGTAACCTACTTAT	386500
1898+1G>A	1	none	synthetic target	ATAAGTAAGGTATCCAAAGACATATCTTCAAAATCTCTTTCTGTAAACATCTAGGTATCCAAAAGGAGAGTCT	904800
2184delA	4	none	invader	CCCCAACTCTCCAGTCTGTTTAAAGATATTTTTTC	393000
2184delA	4	hex	probe/DM	CGCCCGAGGGTGTGTGTCGAGGAGACA	305200
del1507	1	none	invader	GCTTTGATGCGCTGTGATCTATATTCATCATCATTAGGAACACCAAT	509300
del1507	1	hex	probe/DM	CGCCCGAGGAGATATTTCTTAAATGGTGCC	345200
del1507	1	none	synthetic target	GCTGCGCACCAATAGAAAATATCTTTGGTTCCTATGATGAATACAGAAAGCGTCATCAAAAGCATGCC	866600
G85E	4	none	invader	GCCTCTCGGAGTGTTTTCTTGGAGATTTATGTTCTATGT	409100
G85E	4	hex	probe/ER24	ACGAGCGCGGAGAAATCTTTTATATAGGGGTAAAG	431700
G85E	4	none	synthetic target	AGATCTTACCCCTAAATATAAAGAATTCATAGAACAATAATCTCCAGAAAAACATCGCCGAAGGGCATTAT	869300
R117H	3	none	invader	AATCATGCTTCTATGACCCGCGATAACAAGGAGAACT	443800
R117H	3	hex	probe/DM	CGCCCGAGGACTCTATCGCAATTTATCT	304200
R117H	3	none	synthetic target	ATGCCTAGAATATCCGATAGAGTGTCTCTCTGTATCGGGTCTATAGGAAGCTATGATT	681700
R560T	1	none	invader	CATGAATGACATTTACAGCAAAATGCTTGCTAGACCAATAATAGTTATTCACT	595000
R560T	1	hex	probe/ER24	ACGGACGCGGAGGTGCTTAAAGAAATCTTGCT	378100
R560T	1	none	synthetic target	CAACGAGTGAATTTCTTAGCAAGCTGAATACTAATATGGCTAGCAAGCAATTTGCTGTAAATGTGATTCATCTGTAATA	945400
3120+1G>A	2	none	invader	CGCCCGAGGATATGTAATAATAAGTACCCGTAA	496000
3120+1G>A	2	hex	probe/DM	CGCCCGAGGATATGTAATAATAAGTACCCGTAA	397500
3120+1G>A	2	none	synthetic target	AGACATCTTACCGTACTATTTTATACATCTGGATGAAGTCAAAATATGTTAAGAGGCGAGAAAGGTGATCCAAAATTGCTATATC	984000
3659delC	2	none	invader	GAGATGTGGCATCTGTATGTTTGGTTGATCTT	372300
3659delC	2	hex	probe/DM	CGCCCGAGGGTAGSTTTACCTCTGTGG	302800
3659delC	2	none	synthetic target	CATGCCAAGAGGTGAACCTCAAGTCAAGTCAACCAACCACTCAAGAAATGCCAACTCTC	679800
A455E	1	none	invader	CTGTAAAGATATTAATTTCAAGATAGAAGAGGACAGTGTGTGGT	531000
A455E	1	hex	probe/ER24	ACGGACGCGAGAGTGTGCTGGATCCA	298100
A455E	1	none	synthetic target	CCAGTGGATCCGACCTCCCAACTGCTCTTCTTCTATCTTGAAATTAATATCTTCAGG	661000
1078delT	2	none	invader	AGTGCATAGGAAGACAGATAAACAACACCAT	413500
1078delT	2	hex	probe/DM	CGCCCGAGGAGAACCTTGAGAAGAAGAA	355400
1078delT	2	none	synthetic target	AGCTTCTCTCTCGAGGCTCTTGTTGTTTATCTGTGTTCCCTATGCACT	533300
G551D	2	none	invader	CGAGAGAAACAAATATAGTCTTCGAGAAGGTGGAATCACACTGAGTGGAGT	628200
G551D	2	hex	probe/DM	CGCCCGAGGATCAACGACGACAAATTTCT	343800
G551D	2	none	synthetic target	CTTGTAAAGAAATTCCTGCTGGTGTATCCCATCAGCTGATGCCACCTTCTCCAAAGAACTATATTGTTCTTCTCGAAACTT	883100
I148T	1	none	invader	AAATCAAACTAAACATAGCTATCTCATCTGCTCAT	432400
I148T	1	hex	probe/ER24	ACGGACGCGAGGTGTGATGAAGGCCAAA	350200
I148T	1	none	synthetic target	CCATTTTGGCTTCATCACACTGSAATGCACATGAGATAGCTATGTTAGTTTGAATT	643100
N1303K	2	none	invader	CCATATTTCTTGATCACTCCACTGTTGATGGGATCCAAAT	414700
N1303K	2	hex	probe/DM	CGCCCGAGGCTTTTTCTTAAATGTTCCAGAAA	391200
N1303K	2	none	synthetic target	ATTTATTTTCTGGAACATTTAGAAAAGTTGGATCCCTATGAACAGTGGAGTGAATCAAGAAATATGGAAG	867100
711+1G>T	2	none	invader	GCCTTCCAGTTGTATAATTTAACAATAGTGCCTAAAAGATTAATCAATAGGTACATT	
711+1G>T	2	hex	probe/DM	CGCCCGAGGAAATTCATAAATTTGTTCAAGT	
1717-1G>A	2	none	synthetic target	ACCTGAAACAAATTTGATGAATATGATCTATGATTTAATCTTTAGGCAGTGTGTTATTAATATCAACACTGGAAAGGC	927000
1717-1G>A	3	none	invader	GCCTTCAAAATTCAGATGAGCATATAAAGTGACTCTGTAATTTCTATTTTGGTAAAT	685000

**Figure 2 cont'd**

[illegible]

Mutation	Exemplary Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	$\varepsilon_{260}$ M <sup>-1</sup> cm <sup>-1</sup>
Internal control	all	none	invader	tgctactcagctctctacacaaagagagatgagagacacaca	503500
Internal control	all	hex	Probe/SNP4b	tccgcgcgcctctgaggaagcaccacattcag	321200
Internal control	all	none	Synthetic Target	tttcagatggctgctctctcagctgctctctctctctctcttagtgtagacacacataaagatcatctt	698200

				Y-tct-X-agg-cgg-ttt-tcc-ggc-tga-gac-ctc-ggc-gcg-hex
all	Hex	DM/FAM		
1, 2	Hex	ER24/FAM		Y-tct-X-agg-cgg-ttt-tcc-ggc-tga-gac-ttc-ggc-ttc-gt-hex
5	Hex	SNP45/Red		Y-tct-X-tca-ccc-ttt-tcc-sca-aga-gaa-gac-acc-cac-a-hex
all	Hex			

X = Quencher = Z28

**Y = Dye = FAM for 1055-48-08 and 1055-48-09 and Y = Z35 (or Redmond Red) for 1055-49-04**

Mutation	Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	$E_{260}^{-1}$ M <sup>-1</sup> cm <sup>-1</sup>
delF508	delF508	Invader	Invader	TGATGACGCTTCTGTATCTATATCATCATAGGAACACA	441500
delF508	delF508	Hex	WT Probe	CGCGCGAGGCAAGATCATATTTCTTTAATGGT	382200
delF508	delF508	Hex	Mut Probe	AGCTGTCGCGACACAATAATTTCTTTAATGGTGCCA	418100
delF508	delF508	Hex	DM/FAM	Y-ctd-X-agg-egg-ift-ttc-ggc-tga-gac-ctc-gtc-agg-hex	347150
delF508	delF508	Hex	Wingtail/Red	Y-ctd-X-icg-ggc-ift-icg-agg-gta-gtc-gga-ggc-hex	390400
delF508	delF508	none	WT Target	TGCTGGCACCATTAAAGAAAATATCATCTTTTGGTGTCTTCCTATGATGAATATAGATACAGAAGGGTCATCAAA	837500
delF508	delF508	none	Mut Target	ATGCTCTGGCACCATTAAAGAAAATATCATCTTTTGGTGTCTTCCTATGATGAATATAGATACAGAAGGGTCATCAAA	828100

Mutation	Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')
2184delA	2184delA	none	invader	CTTCCTTTTTTCCCAAACTCCAGTCTGTTTAAAAAGATTGTTTA
2184delA	2184delA	hex	MUT probe/DM	CGGCCGAGGTTGTTTCTGTCCAGG
2184delA	2184delA	hex	WT probe/ER24	ACGGACGGGAGTTTGTCTGTCCAGG

**X = Quencher = Z28**

Y = Dye = FAM for 1055-48-08 and Y = Z35 (or Redmond Red) for 1144-16-02

711+1G-T	2	hex	probeIER24	ACGGACGCGGAGATTCAAATTGTCAGG
3849+10kb	2	hex	probeIER24	ACGGACGCGGAGTGAAGACACCCCTGAA

**Fig. 3**

**A**

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
2789+5G>A	26mix	3.94	4.69	1.19
R1162X	29	3.42	2.18	0.62
R347P	15	3.38	4.60	1.36
G85E	21	3.62	2.55	0.70
R560T	9	3.30	2.47	0.75
delI507	1	3.16	1.98	0.63
1898+1G>A	111 A2/8	6.23	2.84	0.46
R117H	30	3.46	1.87	0.54
delF508 homo MT	3	3.44	1.14	0.33
WT gDNA	03-243	3.58	1.06	0.30

  

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
2184delA plasmid/internal control syn. Target	plasmid/syn. Target	4.67	3.65	0.78

**B**

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
A455E	8	3.26	2.88	0.88
3659delC	14	3.38	2.36	0.68
N1303K	16	3.92	2.11	0.54
3120+1G>A	6	3.84	2.45	0.64
G551D	20	3.44	2.04	0.59
WT gDNA	03-243	3.74	1.00	0.27

  

I148T/Internal control	syn. target	4.35	5.08	1.17
1078delT/Internal control	syn. target	4.44	4.97	1.12

**C**

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
711+1G>T	2	3.95	2.82	0.71
W1282X	19	4.44	2.16	0.49
1717-1G>A	28	4.87	2.19	0.45
3849+10kbC>T	5	3.82	2.48	0.65
WT gDNA	03-243	4.67	1.10	0.24

**D**

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
621+1G>T	11	4.23	2.05	0.49
G542X	18	3.40	2.83	0.81
R553X	7	4.53	3.27	0.72
R334W	22	3.72	2.79	0.75
WT gDNA	03-243	4.18	1.14	0.27